ADDITIONAL EXPERIMENT

Test Example 2

In order to monitor the leakage of UCN-01 encapsulated in liposomes in human AGP-containing rat plasma (human AGP: 0.5 mg/mL) with the lapse of time, 0.2 mL of the UCN-01-containing liposome suspension prepared in Comparative Example 4 (see the next page) was mixed with 0.8 mL of distilled water. To 0.05 mL of the resultant liquid mixture, 4.95 mL of the rat plasma containing 0.5 mg/mL human AGP was added and mixed to obtain a liquid sample. The following operation was carried out in the same manner as in Test Example 1 in the present application.

The result is shown in Table 2.

Table 2: Remaining ratio (%) of UCN-01 in liposomes

		UCN-01 remaining ratio (%)
Comparative Example 4	Immediately after mixing	86
	After 3 hours	0

Table 2 shows that the liposomes comprising cholesterol and having an average particle size of 192 nm (see Comparative Example 4) failed to inhibit the leakage of a drug encapsulated in the liposomes in the presence of biological components.

Comparative Example 4

To 0.968 g of hydrogenated soybean phosphatidylcholine, 0.257 g of cholesterol and 0.276 g of PEG-DSPE was added an appropriate amount of $CHCl_3$, followed by dissolving the mixture of lipids in $CHCl_3$ and drying the lipids as a thin film by rotation under reduced pressure. To the lipid film was added 25 mL of a 100 mmol/L citrate buffer (pH 4.0), followed by shaking under stirring with a vortex mixer. After the suspension was frozen and thawed three times, the suspension was passed through a polycarbonate membrane filter (0.4 μm) 4 times at room temperature, and further passed through a polycarbonate membrane filter (0.2 μm) 14 times at room temperature. Then, a 100 mmol/L citrate buffer was added thereto to give a liposome suspension having a concentration of the sum of hydrogenated soybean phosphatidylecholine and cholesterol of 31.25 mg/mL. Separately, 3 mg of UCN-01 was taken and 4.8 mL of the liposome suspension prepared above was added thereto. The pH of the resultant mixture was adjusted to approximately 8 by adding an appropriate amount of a 1 mol/L aqueous sodium hydroxide solution. Then, distilled water was added thereto to give a total volume of 6 mL. UCN-01 was encapsulated in liposomes at room temperature.

The average particle diameter of the liposomes measured by the DLS method was 192 nm.

Comparative Example 5

To 1 g of hydrogenated soybean phosphatidylcholine was added 10 mL of a 100 mmol/L citrate buffer (pH 4.0), followed by shaking under stirring with a vortex mixer for 1 hour at 70°C. Then, a 100 mmol/L citrate buffer was added thereto to give a liposome suspension having a concentration of hydrogenated soybean phosphatidylecholine of 62.5 mg/mL. Separately, 10 mg of UCN-01 was taken and 8 mL of the liposome suspension prepared above was added thereto. The pH of the resultant mixture was adjusted to approximately 8 by adding an appropriate amount of a 1 mol/L aqueous sodium hydroxide solution. Then, distilled water was further added thereto to give a total volume of 10 mL. UCN-01 was encapsulated in liposomes at 70°C.

The average particle diameter of the liposomes measured by the DLS method was 1883 nm.